REMARKS

Claims 66-72 and 84-91 are all the claims pending in the application.

Claims 67-70, 84-85 and 89-90 have been canceled herein. Claims 105 to 108 have been added herein. While Applicants do not agree with the Examiner's position on each of the rejections of the claims, the claims are being amended herein to further the prosecution of the application. After entry of this amendment, claims 66, 71, 72, 86-88, 91 and 105 to 108 will be pending.

Support for the amendment to claim 66 is as follows. The recitation of a DnaG primase fragment represented by amino acids 380 to 599 finds support in Figure 10 which shows many fragments of STAAU_R9 (DnaG primase) that bind to phage polypeptide. In particular, the fragment comprising amino acids 380-599 is shown. The recitation of "binds a polypeptide comprising SEQ ID NO:4" is supported at page 11, lines 10-14, and page 14, lines 22-23, where it is indicated that SEQ ID NO:4 is the 96 ORF 78 polypeptide.

Support for the amendment to claim 72 to recite "said first polypeptide comprises SEQ ID NO: 6" may be found at page 11, line 29 to page 12, line 6 and particularly Figure 10 where SEQ ID NO:6 is shown to bind to the 96 ORF 78 polypeptide (SEQ ID NO:4). Support for the amendment to recite "comprises SEQ ID NO:4" as the bacteriophage polypeptide that binds to SEQ ID NO:6 is also found at page 11, line 29 to page 12, line 6, and at page 14, lines 22-23 (indicating that SEQ ID NO:4 is the 96 ORF 78 polypeptide).

Support for the amendment to claims 86 and 87 to recite "wherein said polypeptide binds a polypeptide comprising SEQ ID NO:4" may also be found at page 11, lines 10-14, and page

14, lines 22-23 (indicating that SEQ ID NO:4 is the 96 ORF 78 polypeptide). Reference to polypeptides having 95% identity as being included among those polypeptides that bind a polypeptide comprising SEQ ID NO:4 may be found at page 12, lines 7-12.

Reference to polypeptides having "at least 97% similarity" in claims 87 and 91 is supported by page 57, lines 14-22 (specifically, line 21).

Support for new claim 105 may be found at page 11, lines 25-26.

Support for new claim 106 may be found at page 11, lines 25-28, in Figure 10 and the accompanying discussion of Figure 10 at page 105, lines 1-14.

Support for new claim 107 may be found at page 57, lines 3-10, in conjunction with claims 86, 87 and 91.

Support for new claim 108 may be found at page 57, lines 10-13, in conjunction with claims 86, 87 and 91.

No new matter has been added. Entry of the amendment is respectfully requested.

I. Claim Objections

At paragraph 9 of the Office Action, claim 91 is objected to as not being grammatically correct.

Applicants include herewith an amendment to claim 91, such that the claim is now grammatically correct. In view of the amendment of the claim, Applicants respectfully request reconsideration and withdrawal of this objection.

II. Rejection of Claims Under 35 U.S.C. §112, First Paragraph

A. At paragraph 12 of the Office Action, claims 66-71, 84-85 and 91 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.

The Examiner states that the rejection is a new matter rejection, in that claims 66 and 70 recite the limitation "less than 566 amino acids of SEQ ID NO:2". The Examiner states that there is no support in the specification for this limitation. The Examiner also states that there is no support in the specification for the limitation "amino acids 1 to 34 of SEQ ID NO:2" as recited in claims 84-85 and 91.

Applicants include herewith an amendment to claim 66, such that the objected expression "less than 566 amino acids of SEQ ID NO:2." has been deleted and that the claim refers instead to a polypeptide fragment represented by amino acids 380 to 599 of SEQ ID NO: 2. Support for this amendment can be found in Figure 10 which shows many fragments of STAAU_R9 (DnaG primase) that bind phage polypeptide, in particular the fragment comprising amino acids 380-599 of SEQ ID NO:2.

As the present amendment cancels claims 67 to 70, 84 and 85, the rejection with regard to these claims is moot.

Claim 91 has been amended to remove reference to "amino acids 1 to 34 of SEQ ID NO:2."

In view of the amendments to the claims, and the points discussed above, Applicants assert that the claims fully comply with the written description requirement, and therefore respectfully request reconsideration and withdrawal of this rejection.

B. At paragraph 13 of the Office Action, the rejection of claims 66-72, 84-87 and 89-91 under 35 U.S.C. §112, first paragraph, as lacking adequate written description support in the specification as filed, has been maintained.

The Examiner states that the rejected claims are drawn to a vast number of widely variant species with respect to both structure and function, comprising a fragment or variant of SEQ ID NO:2 having any activity, including non-functional polypeptides (claims 84-87 and 89-91), having the activity of binding to any bacteriophage polypeptide (Claims 66-67 and 70-72), and/or having an activity selected from the range of biological activities including DNA polymerase activity, RNA primase activity, stimulation of helicase activity of S. aureus DnaC helicase, or stimulation of ATPase activity of S. aureus DnaC helicase. The Examiner concludes that neither full length SEQ ID NO:2 or the fragments thereof shown in Figure 10 are sufficient to represent the entire genus of claimed polypeptides.

Applicants respectfully traverse the Examiner's position for the following reasons.

The claims have been amended such that they now encompass a small group of polypeptides, restricted with respect to both structure and function. Indeed, the polypeptides of claims 66, 71 and new claim 106, are limited by structure in that they encompass only those polypeptides comprising at least amino acids 380 to 599 of SEQ ID NO:2. Similarly, the polypeptides of claim 72 are limited by structure in that they encompass only those polypeptides comprising SEQ ID NO:6 (i.e. amino acids 561 to 599 of SEQ ID NO:2) and those polypeptides comprising SEQ ID NO:4. Additionally, all references to a "variant" have been deleted from all of the claims. Finally, the isolated polypeptides of claims 86, 87, 91 and new claims 107-108,

are limited by structure in that they encompass only those polypeptides having at least 95% identity or 97% similarity to SEQ ID NO:2.

As mentioned above, each of these groups of claims is also limited functionally. The polypeptides comprising fragments of SEQ ID NO:2 (claims 66, 71, 72 and new claim 106) are limited by function in that they must be able to bind to a specific polypeptide (SEQ ID NO:4). Similarly, the isolated polypeptides of claims 86 and 87 are functionally limited in that they must be able to bind to a specific polypeptide (i.e., SEQ ID NO:4). The isolated polypeptides of claim 91 and of new claims 107-108 are also functionally limited in that they must have one of the activities recited in the claim.

In addition, with respect to the isolated polypeptides of claims 86, 87, 91 and new claims 107-108 encompassing polypeptides having at least 95% identity or 97% similarity to SEQ ID NO:2 (i.e. DnaG primase homologues), the Applicants respectfully submit that the skilled artisan would know where changes could be made without affecting the activity of the reference polypeptide (SEQ ID NO:2).

Firstly, the Applicants refer the Examiner to **Appendix I** submitted herewith showing a multiple sequence alignment between the *S. aureus* primase ("R9") of the instant application (SEQ ID NO: 2) and primase from *E. coli* (GenBank Accession No. 0902269A (Nov. 27, 1996)), *B. subtilis* (Acc. No. CAB14451 (Nov. 20, 1997)), *Strep. pneumoniae* (Acc. No. AAK75185 (Aug. 31, 2001)) and *B. stearothermophilus* (Acc. No. AAD20315 (March 15, 1999)). In addition, the Applicants refer the Examiner to the sequence alignment provided with the Office Action dated Jan. 5th 2004 showing conserved residues between the *S. aureus* primase of the

instant application (SEQ ID NO: 2) and the primase from *S. epidermis* (US 6,380,370 to Lynn Doucette-Stamm et al.). Also, the Examiner may refer to Figure 2 of Podobnik *et al.*, J. Mol. Biol. (2000), 300:353-362 (see IDS filed on April 5, 2004) showing the sequence alignment of the catalytic domain (~ AA 115 to 420) of six (6) additional bacterial DnaG primases. At the time of the invention, the skilled artisan could thus have easily prepared such alignments to determine the location of conserved residues, and regions of the protein likely required for activity.

Secondly, as evidenced by the non-exhaustive list of scientific documents discussed hereinafter (see the IDS filed on April 5, 2004 for copies of those documents), the state of the art supports a relatively high level of predictability for domains important for bacterial DnaG primase activity. Indeed, as discussed below, there was sufficient knowledge in the art at the time of the invention of the regions of DnaG primases required for activity.

(i) Ohnishi K. (Nucleic Acids Symp. Ser. (1985), 16:253-256) indicates that the C-terminal domain of the *E. coli* DNA primase is strongly homologous to the C-terminal domain of the RPase beta subunit and to the RPase alpha, suggesting a common ancestor.

(ii) Tougu K. et al. (J. Biol. Chem. (1994) 269(6):4675-82) demonstrate that the N-terminal **49 kDa** domain **from *E. coli* DNA primase is required for catalytic activity** whereas the **carboxy-terminal domain of 16 kDa is required for functional interaction with DnaB (helicase)**.

(iii) Sun et al. (PNAS (1994) 91:11462-66) have produced **mutants of *E. coli* DNA primase lacking 10 and 40 C-terminal amino acids** and having the same pRNA activity as wild-type primase.

(iv) Tougu and Marians (J. Biol. Chem. (1996) 271(35):21391-7) have produced a **mutant *E. coli* DNA primase enzyme fully active as a primase.**

(v) Sun and Godson (J. Mol. Biol. (1998) 276(4):689-703) have identified the DNA sequences required for binding *E. coli* primase.

(vi) Aravind L. et al. (Nucleic Acid Research (1998), 26(18):4205-13) have shown that type IA and II topoisomerases, OLD family nucleases, RecR proteins and DnaG-type primases all contain **a common domain designated Toprim (topoisomerase-primase domain).** This domain consists of approximately 100 amino acids and has two conserved motifs. Site-directed mutagenesis supports an important role for one of these two motifs in catalysis.

(vii) Pan H. et al. (Biochim. Biophys. Acta (1999), 1444(3):429-33) describe the cloning, expression and purification of ***Bacillus stearothermophilus* DNA primase and crystallization of the zinc-binding domain.**

(viii) Sun et al. (J. Bacteriol. (1999) 181(12):3761-7) demonstrated by mutagenesis **only Lys241, and not Lys211 and Lys229, is part of the catalytic center of *E. coli* primase.**

(ix) Keck et al. (Science (2000) 287(5462):2482-86) show **a high resolution crystal structure of *E. coli* primase,** including the catalytic core of the protein.

(x) Podobnik (J. Mol. Biol. (2000) 300(2):353-62) obtained the **crystal structure of *E. coli* primase** and showed that the Toprim domain of the protein is strikingly similar in its structure to that of corresponding domains in DNA topoisomerases.

(xi) Bird et al. (Biochemistry (2000) 39(1):171-82) have shown that the C-terminal domain of *Bacillus stearothermophilus* DNA primase interacts with DnaB (helicase) by making systematic truncations using limited proteolysis and PCR mutagenesis.

(xii) Frick and Richardson (Annu. Rev. Biochem. (2001) 70:39-80) provide an **extensive review of DNA primase structures and functions**.

In view of the above, Applicants submit that the skilled artisan could have easily prepared homology alignments of DnaG primases to determine the location of conserved residues and/or domains and that the literature provided ample information concerning regions of DnaG primases required for bacterial primase activity. Thus, those skilled in the art at the time of the invention would have had an excellent expectation of success for obtaining the claimed homologues with desired primase activity.

In view of the amendments to the claims, the claims are now drawn to a small number of narrowly divergent species with respect to both structure and function, comprising a specific fragment of SEQ ID NO:2, having the specific activity of binding to one particular bacteriophage polypeptide (SEQ ID NO:4), and/or having an activity selected from a limited group of similar biological activities including DNA polymerase activity, RNA primase activity, stimulation of helicase activity of *S. aureus* DnaC helicase, or stimulation of ATPase activity of *S. aureus* DnaC helicase.

Further in view of the amendments to the claims, and the points discussed above, Applicants respectfully assert that the claims have adequate written description support in the specification as filed, and therefore request reconsideration and withdrawal of this rejection.

C. At paragraph 14 of the Office Action, the rejection of claims 66-72, 84-87 and 89-91 under 35 U.S.C. §112, first paragraph, as being non-enabled, has been maintained.

The Examiner states that while the specification is enabling for the isolated polypeptide of SEQ ID NO:2, it is not enabling for all bacterial polypeptide fragments or variants of SEQ ID NO:2, having the ability to bind a bacteriophage polypeptide. The Examiner concludes that undue experimentation would be required to make and/or use the entire scope of the claimed invention.

Applicants respectfully traverse the Examiner's position for the following reasons.

As fully discussed above with regard to the written description rejection, and in view of the amendments to the claims which now encompass a limited number of polypeptides, Applicants assert that the specification provides a representative number of species of the claimed genus, and that the genus is well-defined in both structural and functional terms. Given the knowledge of the skilled artisan, and the disclosure in the specification, the skilled artisan would clearly be enabled to make and/or use the entire scope of the claimed invention without undue experimentation.

More particularly, with respect to claim 72 encompassing the shortest functional polypeptide of SEQ ID NO: 2 (i.e. amino acids 561-599 represented by SEQ ID NO: 6), the Applicants respectfully note that the claim is restricted to two specific polypeptides which are

clearly defined, both structurally (SEQ ID NOs) and functionally (binding activity). Applicants further emphasize that the only essential function that is required for the claimed polypeptides is mutual binding. Any additional function (e.g. primase activity) is absent from the claim since such additional function or activity is totally unnecessary.

Applicants submit that they have described a sufficient number of species to enable the genus of polypeptides encompassed within the claim (i.e., those having the ability to bind to the polypeptide of SEQ ID NO:4, and comprising SEQ ID NO:6). Indeed, as described in pages 105 to 108 of the specification, Applicants have prepared **N-terminal GST tagged polypeptides** of full-length protein of SEQ ID NO:2 and of the **fragment** comprising amino acids 561-599 **(SEQ ID NO: 6)**. Both the GST-tagged full length polypeptide and the GST-tagged fragment of SEQ ID NO: 6 bound the polypeptide of SEQ ID NO:4, as shown by affinity chromatography (Far Western, see line 21, page 107 to line 27, page 108) and by surface plasmon resonance (see line 28, page 108 to line 12, page 109).

Applicants have also made additional fusions proteins for two separate yeast two-hybrid studies, described in pages 103 to 105 of the specification. For these experiments, different combinations of fusion proteins were made, **these fusion proteins having either the yeast Gal4 activation domain or the yeast Gal4 DNA binding domain**. More particularly, as shown in Figure 10, Applicants have cleaved the polypeptide of SEQ ID NO: 2 to produce **thirteen tagged fragments** and have clearly demonstrated that the polypeptide of SEQ ID NO: 6 (i.e. amino acids 561-599) is the minimal region of SEQ ID NO:2 still able to bind to the

bacteriophage polypeptide of SEQ ID NO:4 and that, of course, larger fragment also possess the desired binding activity (six of such larger fragments are shown in Figure 10).

Therefore, Applicants submit that, until proof to the contrary, it is reasonable to assume that ANY polypeptide (including recombinant polypeptides) comprising the minimal domain of SEQ ID NO:6 would also possess the claimed activity of binding the polypeptide of SEQ ID NO:4 .

In view of the above, Applicants respectfully submit that the genus of polypeptides that each comprises SEQ ID NO: 6 is enabled by the numerous species disclosed in the present application and that it is Examiner's burden to prove otherwise. Because production of recombinant polypeptides is a routine procedure, those skilled in the art could prepare additional functional polypeptides comprising SEQ ID NO: 6 without undue experimentation (see the instant application at page 79, line 20 to page 80, line 6). Finally, Applicants point out that the genus of polypeptides that each comprises SEQ ID NO: 6 as encompassed by claim 72 **must possess the activity of binding the polypeptide of SEQ ID NO:4**, and therefore, it would be legally improper to reject claim 72 on the basis the specification fails to disclose polypeptides which are not claimed.

In view of the amendments to the claims, and the points discussed herein and above, Applicants assert that the claims are fully enabled, and therefore respectfully request reconsideration and withdrawal of this rejection.

III. Rejection of Claims Under 35 U.S.C. §102

At paragraph 15 of the Office Action, the rejection of claims 66-71, 87 and 91 under 35 U.S.C. §102(b), as being anticipated by O'Donnell et al. (WO 99/37661, published July 29, 1999), has been maintained.

The Examiner states that the cited claims are rejected because O'Donnell et al. teaches a polypeptide encoded by the *S. aureus dnaG* gene with 93.5% identity to SEQ ID NO:2, and 98.1% similarity to SEQ ID NO:2.

Applicants note that claim 66 has been amended to encompass only specific polypeptides comprising amino acids 380 to 599 of SEQ ID NO:2. Any reference to a "variant" has been deleted from the claims. Furthermore, claim 66 and claims dependent therefrom recite an activity of the polypeptides (binding to the polypeptide of SEQ ID NO:4).

As shown by the Examiner in **Appendix A** provided with the instant Office Action, an alignment of the sequence disclosed by O'Donnell and SEQ ID NO:2 of the present invention shows many differences in the range of amino acids 380-599. Accordingly, O'Donnell does not disclose the specific polypeptides of claim 66 comprising amino acids 380-599 of SEQ ID NO:2 (nor amino acids 229 to 599 of claim 71). Nor does O'Donnell teach or suggest that the polypeptides described therein can bind a bacteriophage polypeptide, let alone the polypeptide of SEQ ID NO:4. Accordingly, the polypeptides of the rejected claims, comprising amino acids 380-599 of SEQ ID NO:2, and having the ability to bind the polypeptide of SEQ ID NO:4, as recited in claims 66 and 71 (and new claim 106), are novel and not anticipated by O'Donnell.

As to claims 87 and 91 (and new claims 107-108), Applicants again include herewith **Appendix II**, demonstrating that the polypeptide of SEQ ID NO:2 and the polypeptide of

O'Donnell only share 91% identity and 92% similarity over the entire length of SEQ ID NO:2. As claims 87 and 91 (part (b)) (and new claims 107-108) recite a minimum identity of 95% and a minimum similarity of 97% over the entire length of SEQ ID NO:2, respectively, the polypeptide of O'Donnell does not anticipate these claims. Furthermore, as with claims 66 and 71 discussed above, O'Donnell does not teach or suggest that the polypeptide described therein can bind a bacteriophage polypeptide, let alone the polypeptide of SEQ ID NO:4.

In view of the comments above and the amendments to the claims, Applicants respectfully assert that the disclosure of O'Donnell does not teach each element of the claimed invention and therefore does not anticipate the rejected claims.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

IV. Rejection of Claims Under 35 U.S.C. §103

At paragraph 17 of the Office Action, the rejection of claims 66-71, 84-85, 87 and 89-91 under 35 U.S.C. §103(a), as being unpatentable over Benton et al. (USP 6,037,123), in view of Burgett et al. (USP 6,162,617) and Harbarth et al. (*Arch. Intern. Med.*, 1998), has been maintained.

The Examiner states that Benton et al. teaches a nucleic acid (SEQ ID NO:34; clone pMP109) isolated from *S. aureus* encoding a polypeptide sharing 93.25% similarity to SEQ ID NO:2. As correctly pointed out by the Examiner, "Benton et al. do not teach a polypeptide encoded by their nucleic acid." Burgett et al. teaches cloning of the DnaG gene of *Streptococcus pneumoniae* and teaches expressing the novel protein encoded by the *dnaG* gene for use in

screening for novel antibiotics. Harbarth et al. is merely referred to by the Examiner as a reference showing that *S. aureus* is known to cause bacterial infections in the human population.

The Examiner further notes that the polypeptide encoded by the polynucleotide of Benton et al. is "93% identical and 96% similar to the full length of the polypeptide of SEQ ID NO:2" at page 20, line 15, of the Office Action.

Applicants first note that they mistakenly suggested at page 25, line 12, of the Amendment filed April 5, 2004 that Benton et al. discloses a polypeptide. Applicants agree with the Examiner's position that "Benton et al. do not teach a polypeptide encoded by their nucleic acid."

Next, as discussed above with regard to the rejection of the claims under 35 U.S.C. §102, the claims have been amended to limit the polypeptides comprising a fragment of SEQ ID NO:2 encompassed by claim 66 to those comprising amino acids 380-599 of SEQ ID NO:2, and those that specifically bind to the polypeptide of SEQ ID NO:4. Any reference to a "variant" has been deleted from the claims. Furthermore, the isolated or purified polypeptides of claims 87 and 91 (and new claims 107-108) have been limited to those having a minimum identity of at least 95% and a minimum similarity of at least 97% to SEQ ID NO:2, and those that bind to the polypeptide of SEQ ID NO:4 (claim 87) or have the activities recited in claim 91.

Benton et al. only discloses a polynucleotide. It does not teach or suggest any polypeptides, let alone the polypeptides comprising the specific fragment of SEQ ID NO:2 (amino acids 380-599) as recited in claim 66. Indeed, Applicants refer to **Appendix B**, provided by the Examiner with the instant Office Action. First, Applicants respectively point out to the

Examiner that the translated polynucleotide sequence of Benton et al. includes over its entire length many sequence ambiguities (e.g. M, R, S, W; column 18, lines 51-54 of the specification, explains that the ambiguities were nucleotide positions for which the sequence could not be determined) that would cause many uncertainties with respect to any putative polypeptide encoded by the polynucleotide of Benton et al. One skilled in the art would also have many difficulties in identifying the exact open reading frame of the polynucleotide of Benton et al. since that sequence comprises at lest seven inserts (see nucleotides at positions 749, 765, 793, 839, 846, 1081, and 1461 of **Appendix B**) that would cause several frame shifts.

Furthermore, **Appendix B** shows at least one difference between any putative polypeptide encoded by polynucleotide of Benton et al. and amino acids 380-599 of SEQ ID NO:2 (see codon GAM coding for either an Asp or Glu at nucleotides 1665-8). Similarly, as Benton et al. does not disclose any polypeptides, neither does it teach or suggest any polypeptides that can bind a bacteriophage polypeptide, let alone the polypeptide of SEQ ID NO:4. Accordingly, the polypeptides of the present invention, comprising amino acids 380-599 of SEQ ID NO: 2, and having the ability to bind the polypeptide of SEQ ID NO:4, as recited in claims 66, 67 and 71 (and new claim 106), are not obvious in view of the teachings of Benton et al., Burgett et al. and Harbarth et al., either alone or in combination.

As to claims 87 and 91 (and new claims 107-108), Applicants refer once more to **Appendix B**, provided by the Examiner with the instant Office Action. As shown therein, and as discussed by the Examiner, a polypeptide generated by translating the polynucleotide of Benton et al. is "93% identical and 96% similar to the full length of the polypeptide of SEQ ID NO:2"

(page 20, line 15, of the Office Action). As claims 87 and 91 (part (b)) (as well as new claims 107-108) now recite a minimum identity of 95% and a minimum similarity of 97% over the entire length of SEQ ID NO:2, a polypeptide encoded by the polynucleotide of Benton et al. does not teach or suggest the polypeptides of claims 87 and 91. Furthermore, as with claims 66 and 71 discussed above, Benton et al. does not teach or suggest that a polypeptide encoded by the polynucleotide described therein can bind a bacteriophage polypeptide, let alone the polypeptide of SEQ ID NO:4.

In view of the comments above and the amendments to the claims, Applicants respectfully assert that the disclosures of Benton et al., Burgett et al. and Harbarth et al., either alone or together, do not teach or suggest each element of the claimed invention and therefore do not make the rejected claims obvious.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

V. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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Date: September 1, 2004

APPENDIX I

Multiple sequence alignment between DnaG primases from:

S. aureus (R9)	SEQ ID NO:2
E. coli	0902269A (Nov 27/96)
B. subtilis	CAB14451 (Nov 20/97)
S. pneumoniae	AAK75185 (Aug 31/01)
B. stearothermophilus	AAD20315 (Mar 15/99)

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R9                      --LRIDQSIINEIKDKTDILDLVSEYVKLEKRGRNYIGLCPFHDEKTPSFTVSEDKQICH
E.coli                  MAGRIPRVFINDLLARTDIVDLINARVKLKKQGKNFHACCPFHNEKTPSFTVNGEKQFYH
B.subtilis              MGNRIPDEIVDQVQKSADIVEVIGDYVQLKKQGRNYFGLCPFHGESTPSFSVSPDKQIFH
S.pneumoniae            ---MVDKQVIEEIKNNANIVEVIGDVISLQKAGRNYLGLCPFHGEKTPSFNVVEDKQFYH
B.stearothermophilus    MGHRIPEETIEAIRRGVDIVDVIGEYVQLKRQGRNYFGLCPFHGEKTPSFSVSPEKQIFH
                            :    :: :   .:*::::.  :.*:: *:*: . ****.*.****.*  :**: *

R9                      CFGCKKGGNVFQFTQEIKDISFVEAVKELGDRVNVAVDIEATQSNSNVQIASD-DLQMIE
E.coli                  CFGCGAHGNAIDFLMNYDKLEFVETVEELAAMHNLEVPFEAGSGPS--QIERHQRQTLYQ
B.subtilis              CFGCGAGGNVFSFLRQMEGYSFAESVSHLADKYQIDFP-DDITVHSGARPESSGEQKMAE
S.pneumoniae            CFGCGRSGDVFKFIEEYQGVPFIEAVQILGQRVGIEVEKPLYSEQ---KSASP-HQALYD
B.stearothermophilus    CFGCGAGGNAFTFLMDIEGIPFVEAAKRLAAKAGVDLSVYELDVRGRDDGQTDEAKAMTE
                        ****   *:.: *  : .   * *:.. *.    : .        .           : :

R9                      MHELIQEFYYYALTKTVEGEQALTYLQERGFTDALIKERGIGFAPDSSHFCHDFLQKKGY
E.coli                  LMDGLNTFYQQSLQQP-VATSARQYLDKRGLSHEVIARFGLVLRPPAGQRLKRFGGNPEN
B.subtilis              AHELLKKFYHHLLINTKEGQEALDYLLSRGFTKELINEFQIGYALDSWDFITKFLVKRGF
S.pneumoniae            MHEDAAKFYHAILMTTTMGEEARNYLYQRGLTDEVLKHFWIGLAPPERNYLYQRLS-DQY
B.stearothermophilus    AHALLKRFYHHLLVHTKEGQAALDYLQARGWTKETIDRFEIGYAPDAPDAAAKLLESHSF
                               **   *  .  .  *  **  ** :.  : .  :       .       .

R9                      DIELAYEAGLLSRNEENFSYYDRFRNRIMFPLKNAQGRIVGYSGRTYTG---QEPKYLNS
E.coli                  RQS-LIDAGMLVTNDQGR-SYDRFRERVMFPIRDKRGRVIGFGGRVLGN---DTPKYLNS
B.subtilis              SEAQMEKAGLLIRREDGSGYFDRFRNRVMFPIHDHHGAVVAFSGRALGS---QQPKYMNS
S.pneumoniae            REEDLLDSGLFYLSDAN-QFVDTFHNRIMFPLTNDQGKVIAFSGRIWQKTDSQTSKYKNS
B.stearothermophilus    SLPVMEKAGLLTKKEDGR-YVGRFRNRIMFPIHDHRGETVGFSGRLLGE---GHPKYVNS
                              .:*::   : .    . *::*:***: : :*  :.:.**         .** **

R9                      PETPIFQKRKLLYNLDKARKSIRKLDEIVLLEGFMDVIKSDTAGLKNVVATMGTQLSDEH
E.coli                  PETDIFHKGRQLYGLYEAQQDNAEPNRLLVVEGYMDVVAVAQYGINYAVASLGTSTTADH
B.subtilis              PETPLFHKSKLLYNFYKARLHIRKQERAVLFEGFADVYTAVSSDVKESIATMGTSLTDDH
S.pneumoniae            RSTAIFNKSYELYHMDRAKRSSGKASEIYLMEGFMDVIAAYRAGIENAVASMGTALSREH
B.stearothermophilus    PETPVFRKGAILYHFHAARVPIRKRQEALLVEGFADVISAAQAGIDYAIATMGTSLTEEQ
                         .* :*.*   ** :  *:    : ..  :.**: **      .:.  :*::**  : ::

R9                      ITFIRKLTSNITLMFDGDFAGSEATLKTGQNLLQ---QGLNVFVIQLPSGMDPDEYIGKY
E.coli                  IQLLFRATNNVICCYDGDRAGRDAAWRALETALPYMTDGRQLRFMFLPDGEDPDTLVRKE
B.subtilis              VKILRRNVEEIILCYDSDKAGYEATLKASELLQK---KGCKVRVAMIPDGLDPDDYIKKF
S.pneumoniae            VEHLKRLTKKLVLVYDGDKAGQAATLKALDEIG-----DMPVQIVSMPDNLDPDEYLQKN
B.stearothermophilus    ARILRP-CDTITICYDGDRAGIEAAWAAAEQLSA---LGCRVKVASLPNGLDPDEYIRVY
                           :    . :   :*.* **  *:  : :         .  : .  :*.. ***  :
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R9                      GNDAFTAFVKNDKKSFAHYKVSILKD-EIAHNDLSYERYLKELSHDISLMKSSILQQKAL
E.coli                  GKEAFEARME-QAMPLSAFLFNSLMPQVDLSTPDGRARLSTLALPLISQVPGETLRIYLR
B.subtilis              GGEKFKNDIIDASVTVMAFKMQYFRKGKNLSDEGDRLAYIKDVLKEISTLSGSLEQEVYV
S.pneumoniae            GPEDLAYLLTKTRISPIEFYIHQYKP-ENSENLQAQIEFLEKIAPLIVQEKSIAAQNSYI
B.stearothermophilus    GGERFAGEAG-CRRPLVAFKMAYLRRGKNLQHEGERLRYIDEALREIGKLSSPVEQDYYL
                        * : :     .   .   : .                         *    .   :

R9                      NDVAPFFNVSPEQLANEIQFNQAPANYYPEDEYGGYIEPEPIGMAQFDNLS-RQEKAERA
E.coli                  QELGNKLGILDDSQLERLMPKAAESGVSR------------------PVPQLKRTTMRI
B.subtilis              KQLASEFSLSQESLTEQLSVFSKQNKPADNSGETKTRRAHLTTKARQKRLRPAYENAERL
S.pneumoniae            HILADSLASFDYTQIEQIVN--ESRQVQRQNRMEGISRPTPITMPVTKQLS-AIMRAEAH
B.stearothermophilus    RQLAEEFSLSLSALHEQLSRSQRERTKPREAPDGETARPMLA-----KKLLPAFQNAERL
                        . :.  :        :.:           .        .     .  : :      :

R9                      FLKHLMRDKDTFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNEL
E.coli                  LIGLLVQNPELATLVPPLENLDENKLPGLGLFRELVNTCLSQPGLTTGQLLEHYRGTNNA
B.subtilis              LLAHMLRDRSVIKKVIDRVG-FQFNIDEHRALAAYLYAFYEEGAELTPQHLMARVTDDHI
S.pneumoniae            LLYRMMESP-LVLNDYRLREDFAFATPEFQVLYDLLGQYG------NLPPEVLAEQTEEV
B.stearothermophilus    LLAHMMRSRDVALVVQERIG-GRFNIEEHRALAAYIYAFYEEGHEADPGALISRIPG-EL
                        ::  ::.. .                       .   :     .              ..

R9                      RETLISLEQYNLNDE-PYENEIDDYVNVINEKGQ-ETIESLNHKLREATRIGDVELQKYY
E.coli                  ATLEKLSMWDDIADKNIAEQTFTDSLNHMFDSLLELRQEELIARERTHGLSNEERLELWT
B.subtilis              SQLLSDILMLQVNQE-LSEAELSDYVKKVLNQRNWSMIKEKEAERAEAERQKDFLRAASL
S.pneumoniae            ERAWYQVLAQDLPAE-ISPQELSEVEMTRNKALLNQDNMRIKKKVQEASHVGDTDTALEE
B.stearothermophilus    QPLASDVSLLLIADD-VSEQELEDYIRHVLNRPKWLMLKVKEQEKTEAERRKDFLTAARI
                                    :  .     : :     .           .       :

R9                      LQQIVAKNKERM--
E.coli                  LNQELAKK------
B.subtilis              AQEIVTLNRSLK--
S.pneumoniae            LERLISQKRRME--
B.stearothermophilus    AKEMIEMKKMLSSS
                         :. :  :
```

APPENDIX II

Optimal global alignment

Sequence 1: O'Donnell (WO 99/37661)(572 letters)
Sequence 2: US 10/025,222: SEQ. ID NO:2 (599 letters)

Substitution matrix: blosum62
Gap penalty: - (11 + 1 * (gap length))

Score: 2831
Identical: 555/605 (91%), **Similar:** 560/605 (92%), **Gap:** 39/605 (6%)

```
seq1     1 M---------------------------------IGLCPFHDEKTPSFTVSEDKQICHCF    27
           :                                 ||||||||||||||||||||||||||
seq2     1 LRIDQSIINEIKDKTDILDLVSEYVKLEKRGRNYIGLCPFHDEKTPSFTVSEDKQICHCF    60

seq1    28 GCKKGGNVFQFTQEIKDISFVEAVKELGDRLNVAVDIEATQSNSNVQIASDDLQMIEMHE    87
           ||||||||||||||||||||||||||||||:||||||||||||||||||||||||||||||
seq2    61 GCKKGGNVFQFTQEIKDISFVEAVKELGDRVNVAVDIEATQSNSNVQIASDDLQMIEMHE   120

seq1    88 LIQEFYYYALTKTVEGEQALTYLQERGFTHALIKERGIGFAPDSSHFCHDFLQKKGYDIE   147
           |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
seq2   121 LIQEFYYYALTKTVEGEQALTYLQERGFTDALIKERGIGFAPDSSHFCHDFLQKKGYDIE   180

seq1   148 LAYEAGLLSRNEENFSYYDRFRNRIMFPLKNAQGRIVGYSGRTYTGQEPKYLNSPETPIF   207
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
seq2   181 LAYEAGLLSRNEENFSYYDRFRNRIMFPLKNAQGRIVGYSGRTYTGQEPKYLNSPETPIF   240

seq1   208 QKRKLLYNLDKARKSIRKLDEIVLLEGFMDVIKSDTAGLKNVVATMGTQLSDEHITFIRK   267
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
seq2   241 QKRKLLYNLDKARKSIRKLDEIVLLEGFMDVIKSDTAGLKNVVATMGTQLSDEHITFIRK   300

seq1   268 LTSNITLMFDGDFPGSEATLKTGQHLLQQGLNVFVIQLPSGMHPDEYIGKYGNDAFTTFV   327
           |||||||||||||| ||||||||||:|||||||||||||||| |||||||||||||| ||
seq2   301 LTSNITLMFDGDFAGSEATLKTGQNLLQQGLNVFVIQLPSGMDPDEYIGKYGNDAFTAFV   360

seq1   328 KNHKKSFAHYKVSILKDEIAHNDLSYERYLKELSHDISLMKSSILQQKAINDVAPFFNVS   387
           || ||||||||||||||||||||||||||||||||||||||||||||:||||||||||||
seq2   361 KNDKKSFAHYKVSILKDEIAHNDLSYERYLKELSHDISLMKSSILQQKALNDVAPFFNVS   420

seq1   388 PEQLANEIQFNQAPANYYPEDEYGGYDEYGGYIEPEPIGMAQFDNLSRREKAERAFLKHL   447
           |||||||||||||||||||      ||||||||||||||||||||:||||||||||||
seq2   421 PEQLANEIQFNQAPANYYPE------DEYGGYIEPEPIGMAQFDNLSRQEKAERAFLKHL   474

seq1   448 MRDKDTFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNELRETLI   507
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
seq2   475 MRDKDTFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNELRETLI   534

seq1   508 SLEQYNLNGEPYENEIDDYVNVINEKGQETIESLNHKLREATRIGDVELQKYYLQQIVAK   567
           |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
seq2   535 SLEQYNLNDEPYENEIDDYVNVINEKGQETIESLNHKLREATRIGDVELQKYYLQQIVAK   594

seq1   568 NKERM   572
           |||||
seq2   595 NKERM   599
```